

CHARGE NUMBER: 6906  
PROJECT TITLE: BIOLOGICAL EFFECTS OF SMOKE  
PERIOD COVERED: August 1-31, 1981  
PROJECT LEADER: R. A. Pages  
WRITTEN BY: T. Yu  
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1. E. COLI ( $\lambda$ ) PROPHAGE INDUCTION ASSAY<sup>1</sup>

A systematic evaluation on the prophage inducing activity of CSC base fraction samples derived from 14 cigarette types were performed in the plate test. The experiments were designed to determine the minimal inducing concentration (MIC) of each sample in order to separate the samples on the basis of activity. The results showed that the samples can be separated into four groups: 1) No detectable activity: low tar reference, LTF-3A, 2A1, and ET. 2) The MIC of 2R1, bright + NO<sub>3</sub> and RCB was 0.1  $\mu$ g. 3) The MIC of ERKS, burley, DIET, RKS, and bright was 1  $\mu$ g. 4) the MIC of LTF-2A was 25  $\mu$ g.

2. V79 CHINESE HAMSTER CELL MUTATION ASSAY<sup>2,3</sup>

Cells were obtained from an outside laboratory. Frozen stock cultures have been made in our laboratory for future use. Currently, the growth characteristics of the cells are being studied.

3. YEAST MITOTIC GENE CONVERSION ASSAY<sup>4</sup>

A good working stock culture of strain D<sub>4</sub> was obtained using the procedure recommended by an outside laboratory.<sup>13</sup> Samples (prepared by Project 6908 personnel) of mainstream smoke (MS) and sidestream smoke (SS) derived from 2R1, bright, burley, RCB, and RL reference were tested using the new stock culture. These samples consisted of materials collected in impaction traps and on Cambridge pads. In addition, the SS samples were generated and collected under both static burning (SB) and static/dynamic burning (S/D) conditions.<sup>5</sup> The experimental data are being analyzed, particularly with respect to water content, which ranged from 8 to 80% in these samples. SS samples contained much higher water content than MS samples. The results and additional experiments of this work will be reported next month.

A culture of *S. cerevisiae* strain D<sub>7</sub> has been added to our culture collection. This strain is suitable for detecting multiple endpoints of genetic effects including mitotic gene conversion and recombination, point mutation and reverse mutation. Experiments are planned to compare the sensitivity of strains D<sub>7</sub> and D<sub>4</sub>.

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4. SALMONELLA/MICROSOME MUTATION ASSAYA. Pyrolysis Studies (with 6908)<sup>6</sup>

The specific activities of burley and bright pyrolysates were determined in TA98 in the presence of microsomes. Pyrolysis was carried out in nitrogen from 400°C to 800°C at 100°C intervals for 12 min. Burley pyrolysate showed a higher level of activity (about two to five fold) than the bright samples. The increase in the specific activity as a function of temperature was similar in both samples, except for the 800°C sample. Burley pyrolyzed at 800°C showed a pronounced increase in the specific activity; whereas the specific activity of bright leveled off at 700°C. More work will be done to substantiate these observations.

B. CSC Base Fraction Activity (with 6908)<sup>6</sup>

The pot residue of a base fraction of burley CSC was partitioned into toluene and 50% methanol. The toluene fraction was subsequently chromatographed on a LH-20 column. It was found that 65-70% of the total activity was located in three of the ten fractions collected. These three fractions represented about 5% of the total weight.

C. Mainstream Smoke (MS) versus Sidestream Smoke (SS) Activity (with 6908)<sup>7</sup>

Samples tested and sample preparations were the same as those described under item 3 of this report. The specific activities of these samples were determined in TA98 plus microsomes. The results showed that MS samples were more active than SS samples. These results are in agreement with previous observations.<sup>8</sup> The rank order of activity is different in MS and SS samples collected under either SB or S/D burning conditions. For the MS IT CSC, the order of specific activity was: burley>RCB>2R1>RL reference>bright. This rank order is similar to results obtained in the past.<sup>9</sup> The specific activities of MS TPM of the five cigarette types studied were either similar to that of MS IT CSC (*i. e.*, RCB) or lower (*i. e.*, burley, and RL reference). For the SS samples, the specific activities of burley, bright, and RL reference collected under S/D burning conditions were higher than those collected under SB conditions. For RCB and 2R1, the SS samples showed comparable levels of activity regardless of the burning conditions.

D. Water Expanded Burley Tobacco<sup>7</sup>

CSC samples, prepared from cigarettes which contained water expanded and control, cased and uncased, burley tobacco (furnished by H. Sun) were tested in TA98 plus microsomes. The results (along with those of an analysis of variance conducted by J. E. Tindall), shown below, indicated that: 1) the cased samples were significantly less active than the corresponding uncased samples; and 2) the expanded samples were significantly less active than the control samples.

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<u>CSC</u>	<u>TA98 Activity</u> <sup>a,b</sup>
Uncased Control	4514 ± 227 ]
Uncased Expanded	3632 ± 558 ]
Cased Control	3012 ± 302 ]
Cased Expanded	2312 ± 336 ]

a) TA98 activity = rev/mg of CSC. The numbers represent the mean ± SD of determinations conducted on four separate CSC preparations from each cigarette type.

b) Samples not within the same bracket are different,  $p < 0.05$ .

#### E. Miscellaneous<sup>7</sup>

One sample was tested at the request of J. E. John.

#### 5. L5178Y THYMIDINE KINASE MUTATION ASSAY<sup>10,11</sup>

The cloning efficiency (CE) of L5178Y cells obtained from ten solvent control (DMSO) samples was  $56 \pm 11\%$ . This is an acceptable value according to the SOP established in our laboratories.<sup>12</sup> The dose response of 2-acetylaminofluorene was established in a separate experiment; the CE values obtained from the solvent and the positive control compound were all acceptable. It was therefore decided that assay work will now be resumed. For next month, the activity of three carbonyl compounds known to be present in large amounts in cigarette smoke will be investigated.

#### 6. REFERENCES

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